

Research with a Defective Lassa Virus

RAC Biosafety Working Group

Biocontainment Recommendations

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Overview (1)

- **Lassa virus is a RG4 agent, and a CDC Select Agent; the wild-type virus causes a fatal hemorrhagic fever for which there are no current vaccines or therapeutic options.**
- **Dr. de la Torre requests review of proposed containment for research with a Lassa Virus (LASV) that is modified to prevent cell to cell transmission in non-complementing cells.**
- **Goals of the research are to:**
 - **Develop a virus that can be manipulated at a lower containment level to facilitate research.**
 - **Use this defective virus to identify compounds that interfere with LASV viral transcription.**
 - **Potentially identify targets for new therapeutics.**

Overview (2)

- **Research with Lassa virus must normally be carried out at BL4 but OBA can reduce containment for certain experiments if there are data to support that decision.**
- **The following slides provide a summary of the proposed research and present containment recommendations of the Biosafety Working Group.**

Isolation of Genomic RNAs of LASV and Generation of LASV cDNA (1)

- LASV is a bi-segmented ambisense RNA virus harboring two genomic segments designated: L (large) and S (small).
- Isolation of purified genomic RNAs of LASV will be done at BL4.
- LASV infected cells will be lysed with guanidinium thiocyanate and phenol so that purified genomic RNAs can be transferred out of BL4 for cDNA synthesis of the individual segments.
- **Purified RNA is not covered under the *NIH Guidelines* as it is not contained in cells.**

Isolation of Genomic RNAs of LASV and Generation of LASV cDNA (2)

- The viral glycoprotein gene (GPC) contained on the S segment cDNA will be deleted and replaced with a heterologous Green Fluorescent Protein (GFP) gene to yield a recombinant defective LASV (rLASV Δ GP-GFP) cDNA construct.
- Plasmids will be constructed to direct the transcription of viral genomic RNA segments (L) and (S Δ GP-GFP) in mammalian cells.
- **Synthesis of cDNA molecules of each of the segments in *E. coli* will be performed at BL2 in accordance with Section III-D-2-a of the *NIH Guidelines*, as these segments individually are irreversibly defective fractions of the genome.**

Additional Biosafety Considerations for Research with LASV cDNA at BL2

- Appropriate biosafety/biosecurity measures should be implemented (see [Points to Consider*](#)):
 - The two genomic cDNAs of LASV should remain separate at all times and be maintained separate from plasmids that can be used to rescue LASV.
 - Only those individuals working on this project should have access to the genomic clones and they should have appropriate biosafety and biosecurity training.
 - An inventory tracking system should be in place.
 - Destruction of the wild-type S segment will add an additional measure of security.
 - Avoid potential cross-contamination with LCMV cDNA constructs that may be present in the laboratory by exercising appropriate spatial and temporal separation of experiments.

* *Points to Consider: http://oba.od.nih.gov/oba/rac/PTC_RG4_MNGV.pdf*

Generation of rLASV Δ GFP-GFP from cDNA

- Rescue of rLASV Δ GFP-GFP virus using complementing cells expressing the deleted GPC gene (a virus encoded membrane glycoprotein).
- The initial rescue attempt will require co-expression of several viral components derived from cDNA constructs in the supporting host cell line.
- **Rescue of defective rLASV Δ GFP-GFP should be performed at BL4 until it is well established that there is no replication competent LASV present in the viral preparation and the defective virus is attenuated compared to wild-type.**

Testing Stability and Infectivity of rLASV Δ GP-GFP

- **Demonstration that rLASV Δ GP-GFP cannot cause disease in an appropriate animal model.**
 - **Testing for avirulence in a guinea pig animal model will provide data on attenuation of rLASV Δ GP-GFP.**
- **Demonstration that replication competent LASV will not be inadvertently created when rLASV Δ GP-GFP is produced in a complementing cell line that provides the missing glycoprotein function.**

Potential Mechanisms for Rescue of Replication Competent (RC) LASV from rLASV Δ GP-GFP

- Rescue of replication competent LASV may occur as a result of:
 - Contamination with Lassa-like agents (e.g. LCMV) that can functionally replace the missing viral glycoprotein.
 - Homologous and/or non-homologous recombination events that although very infrequent, can lead to a recovery of function.
- Homologous recombination during the initial rescue step may be mitigated by creating a stable cell line expressing the viral GPC gene

Testing for Replication Competent rLASV Δ GP-GFP

- **Replication competent virus (RCV) detection:**
 - **Statistically powered to detect a theoretically rare event (e.g. non-homologous recombination leading to RCV rescue) at a pre-determined level of sensitivity.**
 - **Testing should be based on viral growth parameters.**
 - **Detection limits of the biological assay should be determined to avoid false negative results (“spiking” experiments are recommended).**
 - **The number of serial passes for RCV amplification should be based on established experimental parameters.**
- **RCV detection should be carried out at BL4**

Testing for Replication Competent rLASV Δ GP-GFP

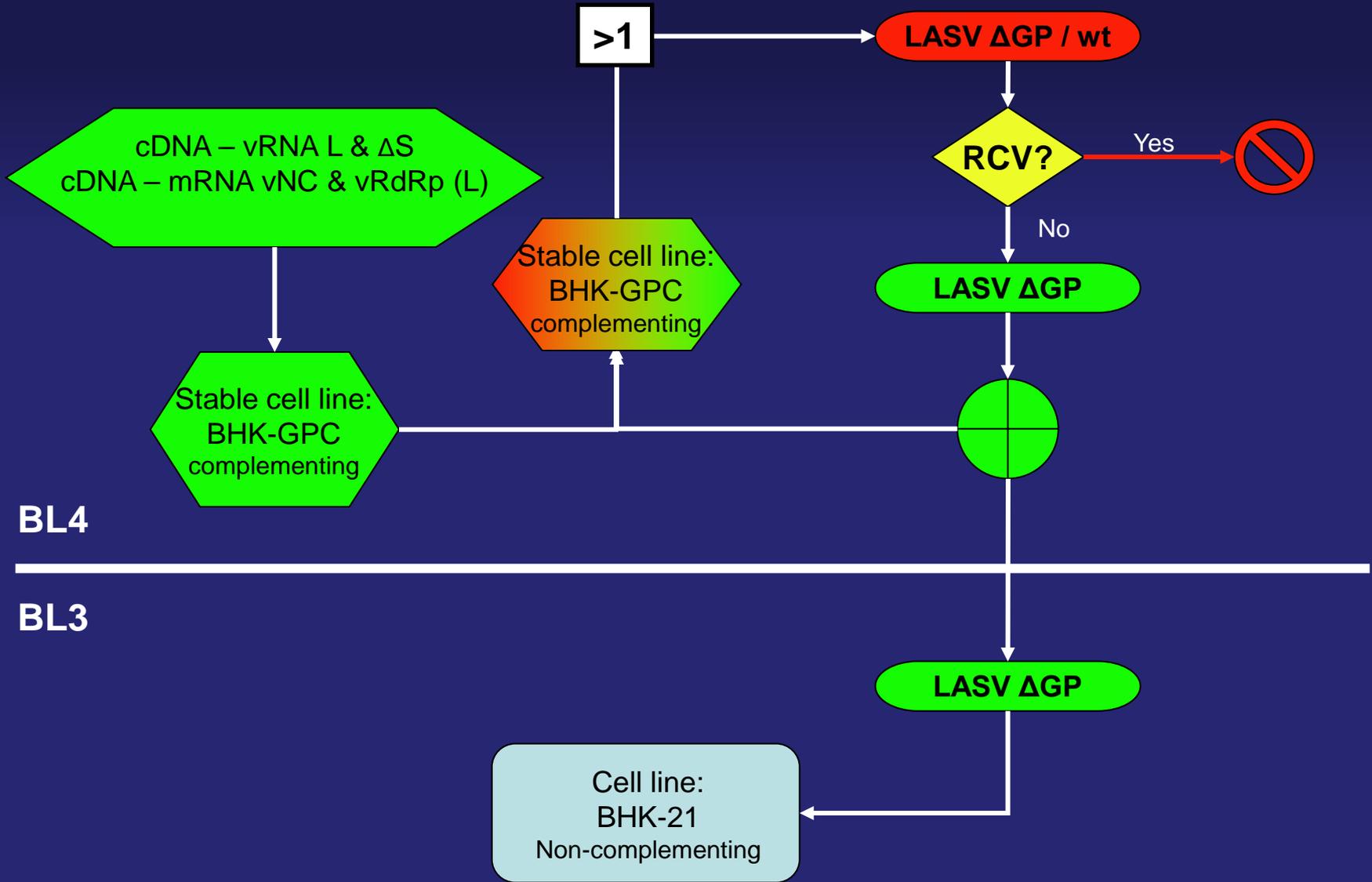
- Batch production of RCV-free rLASV Δ GP-GFP in complementing cells should continue at BL4 until sufficient data are obtained to justify a reduction of containment.
 - Stringency of RCV testing may be reduced if alternate assays of comparable sensitivity are developed (e.g. PCR or other qualified methods).
 - Stability of the rLASV Δ GP-GFP construct should be monitored through successive batch runs.
- **Once sufficient stability data are obtained and a consistent lack of RCV is demonstrated among several consecutive virus batches, it may be safe to produce rLASV Δ GP-GFP at BL3 containment.**

Identifying inhibitors of viral replication

- Once animal testing and RCV testing is complete, RCV-free rLASV Δ GFP-GFP will be removed from BL4 and used to infect non-complementing cells where small molecule or RNAi inhibitors will be tested for their ability to interfere with viral transcription.
 - Procedural controls are recommended to prevent the inadvertent removal of infectious material from BL4 containment.
 - Avoid potential cross-contamination with LCMV virus that may be present in the laboratory by exercising appropriate spatial and temporal separation of experiments involving LASV and LCMV.
 - **Infection of a non-complementing cell line with rLASV Δ GFP-GFP may be performed at BL3.**

Recommendation Summary

CONTAINMENT



High Throughput Screening to Identify Inhibitors of Viral Transcription

- A high-throughput screening device will be used to measure fluorescence thus identifying compounds that significantly reduce GFP expression in rLASV Δ GP-GFP infected cells.
- **Containment may be reduced to BL2 provided that non-complementing cells infected with rLASV Δ GP-GFP are fixed using formaldehyde or glutaraldehyde-based buffers at concentrations that will inactivate LASV.**

Summary of Biosafety Recommendations

- **BL4 containment is recommended for all work with potentially infectious Lassa virus including proof of concept work demonstrating that wild-type LASV cannot be rescued from rLASV Δ GP-GFP through homologous or non-homologous recombination.**
- **Production of new batches of rLASV Δ GP-GFP will continue at BL4 until sufficient data are obtained regarding the stability of this system to allow production at BL3.**

Summary of Biosafety Recommendations

- **BL3 containment is appropriate for experiments involving the stable RCV-free rLASV Δ GP-GFP in a non-complementing cell line.**
- **BL2 containment is appropriate for high throughput screening experiments once the non-complementing cells containing rLASV Δ GP-GFP are completely inactivated.**